Procedure for Extraction & Immunoassay of Chlorpyrifos in Dust

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1 Introduction

Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl], phosphorothioate) is an organophosphate pesticide used in agriculture and the home. Crawling infants and toddlers may be at a much greater exposure risk to track-in dirt and dust-borne pesticides than older children and adults. Therefore, track-in dirt and dust may constitute an important source for assessing exposures. Organophosphates share a common toxic mechanism as an inhibitor of acetylcholinesterase.

We have developed a fast and efficient extraction technique for chlorpyrifos (CPF), using methanol with sonication. A similar method using cyclohexane and sonication to recover polycyclic aromatic hydrocarbons from track-in dirt and house dust has been reported[1], providing a background for this work. In addition, we have developed a technique to reduce the ELISA incubation time from overnight to five min.

2 Sonic Extraction of Chlorpyrifos

Dirt Sample, 200 mg

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Spike with unlabeled and ^{14}C-CPF

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Allow to stand overnight uncovered

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Extract with 10 mL MeOH, sonicate 30 min (repeat once)

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Centrifuge sample 10,000 rpm, 30 s

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1 mL of supernatant is counted

Dislodgement of ^{14}C-CPF from dirt was complete in the first 10 mL extraction (Table 1).

Table 1. Efficiency of Methanol Extraction with Sonication

Sample	Recovery of ^{14}C-CPF 1st Extraction	2nd Extraction	Combined Extracts
Track-in Dirt	114%	17%	107%
Vacuum Cleaner Dust	99%	6%	92%

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The minimum sonication time for extraction was determined. The set-up (panel #2) was using dirt samples spiked with ^{14}C-CPF as the sample. Samples were removed at five min intervals for 30 min and placed into microcentrifuge tubes. Samples were spun for 30 sec and aliquots of 1 mL were then counted. The results are shown in Table 2. Extraction was essentially complete after sonication for 5 min. Extraction was nearly complete at 0 min without sonication, suggesting vortexing, without extraction, could dislodge CPF from the dirt. However, we could not recover more than 93% of the CPF in this manner.

Table 2. Sonication Time and Chlorpyrifos Recovery

Time, min	% ^{14}C-CPF Recovered
0	86
5	97
10	96
15	104
20	96
25	102
30	109

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CPF is readily dislodgeable from leaf foliage by dilute detergent solutions and shaking[2]. We substituted PBST (phosphate buffered saline with 0.05% Tween-20) for MeOH to see if we could eliminate MeOH. The usual track-in dirt spiking with unlabeled and ^{14}C-CPF was performed and allowed to sit uncovered overnight. The next morning it was extracted with 10 mL PBST with a 30 min sonication. As seen in Table 3, chlorpyrifos was only minimally extracted by this procedure.

Table 3. Effect of Sonic Extraction with PBST

Method	Percent Extracted
Immunoassay	25
^{14}C-CPF Count	17

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Results of immunoassay studies (panel #4) were always spurious and it was suspected MeOH might be interfering with the assay. Therefore, various concentrations of MeOH in PBST were spiked with 50 µg/L CPF. The samples were then tested by immunoassay. Results were determined using a standard curve containing no MeOH. The data in Table 4 indicated concentrations of MeOH greater than 5% interfered with the assay for CPF.

Table 4. Effect of Methanol on the Chlorpyrifos ELISA

Percent MeOH	Results, µg/L
0	47
5	47
10	44
15	39
20	34

(Expected = 50 µg/L)

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Using 5% MeOH, sonication times were optimized to extract all chlorpyrifos from track-in dirt. Using the protocol described above we spiked track-in dirt with unlabeled and ^{14}C-CPF and allowed it to sit overnight. Samples were placed in a sonic bath and sampled at five min intervals for 20 min. Table 5 shows the results of this experiment. Using both unlabeled and ^{14}C-CPF, we show that extraction of chlorpyrifos from track-in dirt is essentially complete with 5% MeOH and a 10 min sonication time.

Table 5. Chlorpyrifos Extraction with 5% Methanol

Time, min	ELISA Results,* µg/L	% of Expected Immunoassay	^{14}C-CPF Count**
0	11.5	46	62
5	22.2	89	70
10	24.2	97	101
15	25.4	102	-
20	25.3	101	100

*Expected = 25 µg/L
**Expected = 5680 dpm

7 Immunoassay Study

The indirect ELISA used in this study was that reported by Van Emon, J.M., et al.[2] in a study of foliar dislodgeable residue analysis of chlorpyrifos. The procedure calls for mixing the analyte with equal volumes of the diluted primary antibody (anti-chlorpyrifos) and incubating overnight at 4°C to allow the antigen (CPF) and antibody to fully react. To optimize for speed the analyte-antibody mixture was subjected to sonication, on the basis that the intermolecular collisions of antigen and antibody should be increased by many orders of magnitude over passive interaction at 4°C.

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Standards with primary antibody were subjected to various periods of sonication then assayed. Sonication times of 2, 3, 4, 5, and 8 min produced acceptable standard curves (Figure 1). The 8 minute curve shows that longer than 5 min sonication times did not improve the standard curve. Table 6 shows the similar IC_{50} points of the curves.

Figure 1. Standard Curve Plots and Sonication Times

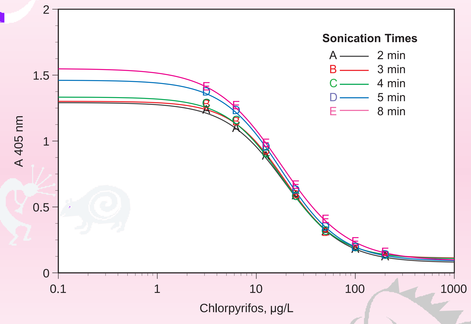

Table 6. Standard Curve IC_{50} and Sonication Time

Time, min	IC_{50}, µg/L
2	17.8
3	16.1
4	16.1
5	16.8
8	17.4

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A commercial formulation of chlorpyrifos (Dursban®) was assayed by the above method versus overnight incubation. The two procedures showed very close agreement (Table 7).

Table 7. Dursban® Assays

Incubation	% Chlorpyrifos
Sonication, 5 min	7.5
Overnight at 4°C	7.6

10 Summary

Chlorpyrifos was seen to be completely dislodged from dust and track-in dirt in a procedure utilizing methanol extraction with sonication. Optimal conditions were 5% methanol in PBST and 10 minutes sonication time in an ultrasonic cleaning bath. This procedure requires only a short centrifugation period in a microcentrifuge which readily pellets dirt and allows easy pipetting of the supernatant fluid. By decreasing the incubation time of primary antibody and analyte from overnight to 5 minutes, the sample preparation and ELISA could be completed in one day. If a large number of samples is to be analyzed the overnight incubation may be more convenient. We believe this procedure will also work with any number of antibodies and will continue to explore this abbreviated assay format.

References

1. Chuang, J.C., *et al.* Development and Evaluation of Monitoring Methods for Polycyclic Aromatic Hydrocarbons in House Dust and Track-In Soil. Batelle Memorial Institute. 1993. Under EPA Contract No. 68-D0-0007, Atmospheric Research and Exposure Assessment Laboratory, U.S. Environmental Protection Agency, MD-44, Research Triangle Park, NC 27711.

2. Van Emon, J.M., *et al.* Foliar Dislodgeable Residue Analysis: A New Scientific Approach to a Regulatory Concern. 1998. Food Technol. Biotechnol. 36, 119-124.